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Entomopathogenic Nematodes from the Coastal Belt of Sri Lanka and their Efficacy in Controlling Termites

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ABSTRACT

The present study was carried out to assess the availability of entomopathogenic nematodes in the north-western coastal belt around Puttalum lagoon, three selected agricultural lands in Gampaha district of Sri Lanka and from the coastal belt along the city of Colombo. Nematodes could only be recovered from the coastal belt along the city of Colombo belonging to *Heterorhabditis* and *Steinernema* spp. One common isolate was identified as *Heterorhabditis indicus* (Poinar). Attempts were also made to examine the relationship between the presence of entomopathogenic nematodes and the texture of the soil. Statistical analysis showed that percentage clay and soil moisture are inversely relate to the recovery of entomopathogenic nematodes. The potential use of *H. indicus* to control two species of subterranean termites, *Odontotermes horni* and *Odontotermis redemanni* (Isoptera: termitidae) was tested in the laboratory. LD_{50} for *O. horni* was 2.8×10^2 or 2.2×10^2 after four or five days of exposure (LD_{50} for *O. redemanni* was 2.3×10^2 or 1.8×10^2) to infective nematodes, respectively.

Keywords: bioassay, biocontrol, *Heterorhabditis indicus*, *Odontotermes* spp., *Steinernema*

INTRODUCTION

Chemical insecticides have been used as the primary means of controlling insects. However, concerns about human health, environmental contamination, as well as development of insect resistance have forced a need for alternative control strategies. Entomopathogenic nematodes are lethal parasites of various life stages of a wide range of host insects. The Heterorhabditidae and Steinernematidae families contain the most widespread insect parasitic nematodes, whose infective juveniles are the only stage that survive outside the host body, in the soil. Nematodes of these two families provide some of the most promising biological control agents of insect pests (Kaya and Gaugler 1993).

The life cycle of both types of nematodes is basically similar. The infection is initiated by a special third stage juvenile or "dauer larva" which is morphologically and physiologically adapted to survive in the environment for a prolonged period (Poinar 1990).

When the infective juveniles locate a host, they enter via cuticle or natural body openings of the host such as the mouth, anus and spiracles. The infective juveniles carry a specific bacterium of the genus *Xenorhabdus* in their anterior intestine which is mutualistically associated with the juvenile. Once the infective juveniles enter the body cavity of the host they release the *Xenorhabdus* into the haemocoel of the host, where the bacterium produces a chemical that destroys any antibacterial proteins of the insect host (Akhurst 1983), and starts to multiply which causes the death of the host within 24-48 hours of the entry of nematodes, by septicaemia (Glazer *et al*. 1991). The death of the host may produce suitable conditions for nematode development, feeding and reproduction. Several generations of the nematode may proceed inside the host body if the nutrients are adequate, before infective juveniles exit the cadaver to seek a new host.

Entomopathogenic nematodes show many desirable characteristics which permits them to be used as efficient pest control agents. Among them, efficacy over a wide host range and various host stages, low production cost, feasibility to be produced under low technology, non toxicity to vertebrates and plants, low toxicity to non-target insects and persistence in natural environments, are most important factors (Woodring and Kaya 1988; Hominick 1990). A large number of trials have been performed to use entomopathogenic nematodes against insect pests in the field. Both failures and successes are encountered in these trials (Poinar 1979; Kaya 1985; Klein 1990). Encouraging results have been obtained for *Steinernema carpocapsae* against the Current borer moth, *Synanthedon tipuliformis* (Miller and Bedding 1982), *Heterorhabditis heliothidis* against Black vine weevil, *Otiorhynchus sulcatus* (Bedding and Miller 1981), *Steinernema feltiae* against Raspberry crown borer, *Pennisetia marginata* (Capinera *et al.* 1986). Several studies have been reported the use of entomipathogenic nematodes to control termites in laboratory assays and in the field. Termites can be grouped as dry-wood, live-wood, arboreal and subterranean according to the type of nests they make (Cranham 1966). In Sri Lanka, promising results have been obtained in controlling live wood tea termite, *Glyptotermes dilatatus* by *Heterorhabditis* isolate D1 (Danthanarayana and Vitarana 1987) and live wood tea termite, *Postelectrotermes militaris* by *S. carpocapsae* and *S. feltiae* (Amarasinghe *et al*. 1994). Subterranean termites, *Coptotermes*, *Reticulitermes*, *Mastotermes*, *Schedorhinotermes*, and *Odontotermes* species are regarded as the most destructive cosmopolitan subterranean termite species (Pearce 1997) and they pose a greater pest threat than any other type of termites, commonly extending under buildings. Several studies have been focused in controlling these termites using entomopathogenic nematodes. For example, *Steinernema riobrave* Cabanillas, Poinar and Raulston (355 strain), *Steinernema carpocapsae* (Weiser) (Mexican 33 strain), *Steinernema feltiae* (Filipjev) (UK76 strain), and *Heterorhabditis bacteriophora* Poinar (HP88 strain) were shown to be capable of infecting and killing three termite species, *Heterotermes aureus* (Snyder), *Gnathamitermes perplexus* (Banks), and *Reticulitermes flavipes* (Kollar) in laboratory sand assays (Yu *et al*. 2006). Infectivity of *S. carpocapsae*, *S. riobrave*, *H. bacteriophora* and *H. indica* against two

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subterranean termites, *Reticulitermes flavipes* and *Coptotermes formosanus* have been reported (Wang *et al.* 2002).

The major problem in using entomopathogenic nematodes in pest control is their long term efficacy and viability. However, experiments have shown that these nematodes are able to survive a prolong period (Koppenhofer *et al.* 1997; Fitters and Griffin 2006). Some countries market commercial products of entomopathogenic nematodes in various formulations such as activated charcoal, alginate capsules, alginate sheets, baits, clay, evapo-retardants, ultraviolet protectants, gel-forming polyacrylamides, absorbent pads, peat, polyether polyurethane sponge and vermiculites to optimize the survival and establishment of these nematodes. Though these products show attractive results, native entomopathogenic nematodes are almost always more successful than foreign species, probably being more adapted to local environmental factors. Native species of *Heterorhabditis* was found more efficient than *Steinernema feltiae* and *S. carpocapsae*, exotic species of entomopathogenic nematodes, in killing the termite, *Postelectrotermes militaris* in Sri Lanka (Amarasinghe and Hominick 1993). Therefore, continuous attention to the isolation and culture of native entomopathogenic nematode isolates is important.

The present study was conducted to determine the availability of steinernematid and heterorhabditid nematodes in the North-western coastal belt of Sri Lanka; to determine the availability of above nematodes in selected agricultural lands in Gampaha district in Sri Lanka; to revisit the documentation on availability of steinernematid and heterorhabditid nematodes in the western coastal belt along Colombo city area of Sri Lanka (Amarasinghe *et al.* 1994) and to document the efficiency of *Heterorhabditis indicus* (Rhabditida: Heterorhabditidae) on the mortality of *Odontotermes horni* and *O. redemanni* (Isoptera: Termitidae).

MATERIALS AND METHODS

Sampling for native entomopathogenic nematodes

Soil samples were collected from the North-western coastal belt around Puttalum lagoon (7° 54′ 18.15″ N, 79° 50′ 41.86″ E), the Western coastal belt along the city of Colombo (6° 56′ 57.17″ N, 79° 50′ 06.38″ E) and three agricultural lands each at Ragama (7° 01′ 21.37″ N, 79° 55′ 10.69″ E), Meerigama (70° 6′ 06.96″ N, 80° 00′ 21.55″ E) and Nittambuwa (7° 06′ 45.05″ N, 80° 00′ 56.80″ E) in Gampaha district in Sri Lanka (**Fig. 1**). Ten sampling sites were selected at each location. Each soil sample was taken to a depth of 10–15 cm using a 250 cc soil corer. Three random samples within a radius of 2-5 m at each site were taken so as to make a composite sample with a volume of approximately 750 g. Composite soil samples were placed in labeled polythene bags and transported to the laboratory in rigifoam thermal boxes. About 200 g each from a composite soil sample was taken to fill three plastic containers. The presence of entomopathogenic nematodes was determined by a baiting technique using five later instar larvae of *Galleria mellonella* (wax moth larvae) (Poinar 1979) which were introduced into each container (250 ml) (**Fig. 2**). The larvae were examined after 3-4 days for their mortality due to nematodes. Any dead cadavers of *G. mellonella* were collected from the containers and modified white traps were operated to extract infective juveniles from the cadavers (**Fig. 3**) separately. Infectivity of the juveniles isolated at each site was rated as low, moderate or high based on the mean number of infectives encountered per cadaver that was less than 5, between 100 and 500 or more than 500 respectively.

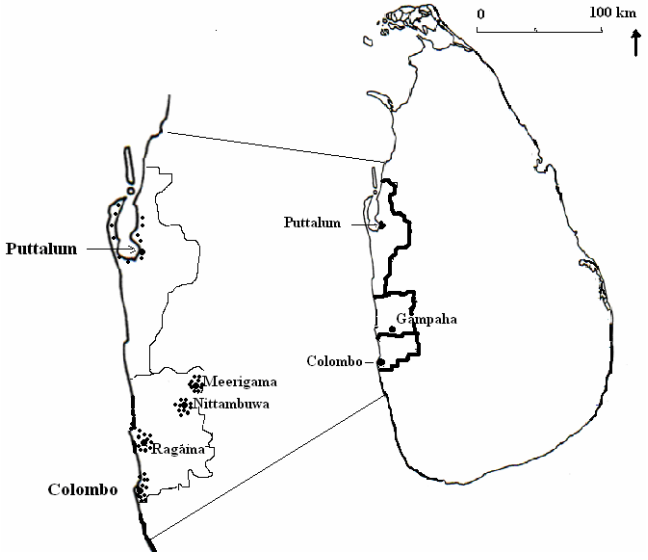

Fig. 1 Map of Sri Lanka showing the sampling locations.

Fig. 2 Showing the arrangement of soil samples for live bait technique to detect entomopathogenic nematodes from soil.

Fig. 3 Modified white trap technique to extract infective juveniles.

Identification of entomopathogenic nematodes

The keys provided by Poinar (1979, 1990, 1993) and Poinar *et al.* (1992) were used in the identification of nematode isolates. Colour changes of the infected cadaver helped to pre-determine whether isolates were heterorhabditids or steinernematids. Identification was confirmed only to the genus level for all native isolates of *Steinernema* by morphometry, colour and shape of external genitalia of adult males.

Analysis of soil characteristics

The percent clay, organic matter and moisture for each soil sample were analysed by a gravimetric method.

Screening assay for efficacy of *H. indicus* on subterranean termites, *Odontotermes horni* and *O. redemanni*

"Worker" stages of both termite species were collected from natural nests located at Nittambuwa (7° 06′ 45.05″ N, 80° 00′ 56.80″ E) in Gampaha district. Bioassay tests were performed in 9 cm diameter Petri dishes lined with moistened tissue paper. Twenty "worker" termites were placed in the Petri dishes with 2 g of evenly distributed saw dust and covered. Dosages of 100, 300, 500, 800 and 1000 nematodes were tested for both termite species. Each dosage for each termite species was replicated 10 times with untreated controls. Termite mortality was recorded daily for 5 days and was confirmed by observing infected juveniles inside cadavers.

Statistical analysis

Probit analysis to estimate LD_{50} and slope of regression were performed using MINITAB 14 statistical package. Two sample t tests were performed to determine significant difference between number of log transformed termites to stabilize the variance from nematode treated against controls. Correlation at the 95% level of significance was performed to relate the availability of entomopathogenic nematodes with soil characteristics after performing ANOVA. Mean data values were compared by student's *t*-tests; data were followed by ± standard error obtained by descriptive statistics.

RESULTS AND DISCUSSION

Sampling carried out at sites located in the western coastal belt along Colombo city was positive for both steinernematid and heterorhabditid entomopathogenic nematodes (**Table 1**). All the other locations were negative for these nematodes. In Sri Lanka, soil samples have been tested for the presence of entomopathogenic nematodes in parts of the country except for the northern, north-western and eastern provinces from 1992 (Amarasinghe *et al*. 1994) and entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) have been recorded along the Western and Southern coastal belts. The present study included an investigation on the availability of entomopathogenic nematodes in the north-western coastal belt around Puttalum lagoon and agriculture lands in Gampaha district in Sri Lanka which has not been covered before. Puttalum area is one of the hottest and the driest and becoming a dry zone of Sri Lanka receiving ~750 mm rainfall and with an average temperature of about 35°C. Even though entomopathogenic nematodes have been recovered in highly saline conditions (Hara *et al.* 1991; Amarasinghe *et al.* 1994) and at 32°C (Amarasinghe *et al.* 1994), we did not find any nematodes under these conditions in the present study in the Puttalum area where the soil temperature was in the range of 35-45°C during sampling times. However, the temperature of the soil does not explain the absence of entomopathogenic nematodes in cultivated lands as these lands and the sampling sites around Colombo were in same range of 28 ± 2°C during sampling.

Table 2 summarizes the mean % moisture, clay (w/w) and organic matter (w/w) at pooled sites at Puttalum coastal belt (n=10), three agricultural lands (n=30) and Colombo coastal belt (n=10). The entomopathogenic nematodes so far not recovered from clay soils may be due to the limitation of their movement and less aeration. Clay content of positive sites in the present study is significantly lower than that of negative sites. There was a significant inverse relationship between the percentage of clay content and the presence of entomopathogenic nematodes in the soil (F=17.23, p=0.000, df=50, R^2=41.79%) at 95% confidence intervals. Similarly there was a negative relationship between soil moisture and the availability of nematodes (F=29.59, p=0.000, df=50, R^2=55.2%) at 95% confidence intervals. Nematodes were continuously recovered during the present study from the dry sandy areas of the Colombo coastal sites. Hara *et al.* (1991) reported that isolation of entomopathogenic nematodes is restricted to areas with lower soil moisture. Kung *et al.* (1990) reported that nematode survival and pathogenicity decreases more rapidly in moist than dry soils. Organic matter content reported in the present study had no any relationship with the availability of nematodes.

It is now evident that entomopathogenic nematodes in Sri Lanka only occur in sandy soils restricted to the coastal belt. However, in other parts of the world these nematodes have been recovered from many other types of soil. For example, they were associated with calcareous soil in England (Hominick and Briscoe 1990), and loam soil in North Ireland (Blackshaw 1988). These nematodes, steinernematid and heterorhabditids have also been found in a varying range of habitats including cultivated lands, fallow lands,

Table 1 Recovery of entomopathogenic nematodes from sampling sites located along the coastal belt in Colombo, Sri Lanka.

Location	Type of soil	Habitat	Presence of entomopathogenic nematode	Density of infective juveniles/ sample
Galleface	Sandy loam	*Ipomoea pescaprae*	negative	-
Kollupitiya	Sandy loam	*I. pescaprae*	*Heterorhabditis* spp.	low
Bambalapitiya 1	Sandy loam	Grass	*Heterorhabditis* spp. and *Steinernema* spp.	low to moderate
Bambalapitiya 2	Sandy loam	*I. pescaprae*	*Heterorhabditis indicus*	moderate
Bambalapitiya 3	Sandy loam	*I. pescaprae*	*H. indicus*	high
Wellawatte 1	Sandy	*I. pescaprae*	*H. indicus*	high
Wellawatte 2	Sandy loam	Grass	*H. indicus*	high
Dehiwala 1	Sandy loam	Grass	*H. indicus*	high
Dehiwala 2	Sandy loam	Grass	*H. indicus*	high
Dehiwala 3	Sandy loam	*I. pescaprae*	*H. indicus*	high

Low: < 5; moderate: 100-500; high: > 500 infectives per *Galleria mellonella* cadaver.

Table 2 Mean % moisture, clay (w/w) and organic matter (w/w) at pooled sites at Puttalum coastal belt, three agricultural lands and Colombo coastal belt, Sri Lanka.

Pooled sites each at	Moisture (%)	Clay (%, w/w)	Organic matter (%, w/w)	Availability of entomopathogenic nematodes
Puttalum coastal belt (*n*=10)	3.84 ± 0.44	15.1 ± 0.82	5.59 ± 0.60	negative
Agriculture lands at Ragama, Meerigama and Nittambuwa (*n*=30)	4.00 ± 0.26	9.23 ± 0.21	4.66 ± 0.49	negative
Colombo coastal belt (*n*=10)	0.67 ± 0.09	3.1 ± 0.74	5.61 ± 0.46	positive
statistics	NS	f=29.22, p=0.000, df=29, r^2=66.06	NS	

Table 3 Comparison of mortality of two species of termites (%±SE) to a series of concentration of *H. indicus* over a period of five days using Student's *t*-test.

Nematode dosage	Termite species	2nd day	3rd day	4th day	5th day
0	*O. redemanni*	1 ± 0.20	2 ± 0.29	2 ± 0.29	3 ± 0.40
	O. horni	1 ± 0.20	2 ± 0.29	2 ± 0.29	2 ± 0.29
	p				0.43
	t				0.685
	df				6
100	*O. redemanni*	6 ± 0.21	8 ± 0.24	14 ± 0.58	18 ± 1.60
	O. horni	2 ± 0.29	4 ± 0.20	8 ± 0.24	14 ± 0.37
	p	2.53	2.53	1.90	1.13
	t	0.039	0.039	0.116	0.301
	df	7	7	5	6
300	*O. redemanni*	30 ± 2.32	38 ± 0.66	53 ± 1.43	66 ± 3.56
	O. horni	21 ± 2.58	33 ± 0.51	48 ± 5.75	56 ± 3.61
	p	2.71	1.18	0.62	1.18
	t	0.035	0.277	0.559	0.292
	df	6	7	6	5
500	*O. redemanni*	56 ± 1.46	76 ± 5.32	85 ± 4.89	100 ± 0.00
	O. horni	44 ± 2.86	58 ± 1.40	76 ± 3.37	91 ± 2.37
	p	1.41	1.87	1.86	
	t	0.207	0.103	0.123	
	df	6	7	5	
800	*O. redemanni*	64 ± 1.86	92 ± 1.51	100 ± 0.00	100 ± 0.00
	O. horni	51 ± 1.75	78 ± 3.40	98 ± 3.24	100 ± 0.00
	p	2.14	4.32		
	t	0.065	0.003		
	df	8	7		
1000	*O. redemanni*	65 ± 1.55	90 ± 2.32	100 ± 0.00	100 ± 0.00
	O. horni	63 ± 3.08	89 ± 1.20	100 ± 0.00	100 ± 0.00
	p	0.33	0.53		
	t	0.754	0.612		
	df	5	6		

Table 4 Probit analysis of LD_{50} of the *H.indicus* for control of *Odontotermes horni* and *O. redemanni* at 95% confidence limit.

Termite species	Exposure time (days)	LD_{50}	Log transformed Probit regression slope	Confidence Interval	
				Lower limit	Upper limit
O. redemanni	2	5.3×10^2	3.91145 ± 0.304378	2.66	2.78
	3	3.2×10^2	3.49953 ± 0.258513	2.46	2.55
	4	2.3×10^2	3.00245 ± 0.228529	2.33	2.42
	5	1.8×10^2	1.97437 ± 0.202530	2.23	2.42
O. horni	2	6.9×10^2	3.67522 ± 0.273062	2.78	2.90
	3	4.1×10^2	3.67213 ± 0.272576	2.57	2.66
	4	2.8×10^2	2.93584 ± 0.241245	2.40	2.49
	5	2.2×10^2	2.21652 ± 0.236834	2.31	2.40

forests, grasslands, roadsides, and sea beeches. For example, they have been recovered from coastal line in eastern China (Qiu *et al*. 2005), Oregon (Liu and Berry 1996), and both isolates have been frequently recovered at the shores of water bodies in the central Rift valley region in Kenya (Mwaniki *et al.* 2008). Soil pH may be another important factor affecting the presence of entomopathogenic nematodes. Kung *et al.* (1990) found that pH values below 4 and above 8 affect the survival and pathogenicity of entomopathogenic nematodes. Soil pH at coastal belt in Sri Lanka remained in a range of 8.0–8.3 and in agricultural lands in a range of 6.3–7.7 which were not unfavourable to entomopathogenic nematodes. Mwaniki *et al.* (2008) showed that the occurrence of *Steinernema* and *Heterorhabditis* isolates clustered at 2–3% carbon and pH 5.3–6.3 with no specific pattern demonstrated from soil types.

The nematode isolates recovered from the coastal belt along the city of Colombo during the present study were comparable to those recovered in 1991 (Amarasinghe *et al*. 1994). The reoccurrence of heterorhabditids and steinernematids in a single coastal site was evident (**Table 1**). The nematode species recorded in highest number of sites and highest density was *Heterorhabditis indicus* and the other isolates recovered did not thrive in laboratory cultures.

Screening assay for efficacy of *H. indicus* on subterranean termites, *Odontotermes horni* and *O. redemanni*

H. indicus was used to test the potential in controlling two subterranean termites in the laboratory. Bio assay of termites using *H. indicus* had a positive result (**Table 3**). Susceptibility as indicated by LD_{50} value estimation varied among two termite species. The number of infectives required to kill termites over a period of five days for *O. horni* and *O. redemanni* indicates that *H. indicus* is more promising against *O. redemanni* than against *O. horni* (**Table 4**). The dose of 1000 nematodes was equally effective on both species of termites throughout their exposure to termites in this study (**Table 4**). However, significant differences were observed at shorter exposure periods when the termites are exposed to doses of 300, and 800 nematodes. Results indicate that the bio-assays are quite promising, capable of 100% mortality in five or less than five days. However, field applications have to be performed to obtain an elaborate picture of the use of *H. indicus* to control *O. horni* or *O. redemanni*. A study conducted by Wang *et al*. (2002) reports that the LD_{50} value for *H. indica* against subterranean termite, *Reticulitermes flavipes* in a Petri dish assay and containers with vermiculite/sand medium were found to be 296 and 264 nematodes per termite. The LD_{50} value for *H. bacteriophora* against the same ter-

mite species in Petri dish assay in the same study was 494 nematodes per termite. The authors also reported that *H. indica* was able to repel termites at high concentrations of above 2000 infective juveniles in both study methods (Wang *et al*. 2002). The length of repellency has varied with the nematode concentration. Authours also reported that nematodes were able to reproduce from termite body. Dampwood termite, *Zootermopsis angusticollis* exhibited a dosage-dependent susceptibility to *Steinernema carpocapsae* (Maxican strain) but, its social behavior was significantly altered in response to this nematode (Wilson-Rich *et al*. 2007).

CONCLUSIONS

Entomopathogenic nematodes have the potential to control termites. The use of native entomopathogenic nematodes has more potential in a pest control programme as they thrive well in native habitats. However, innovative methods have to be used in field application of such nematodes in controlling termites, such as baits or attractants.

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